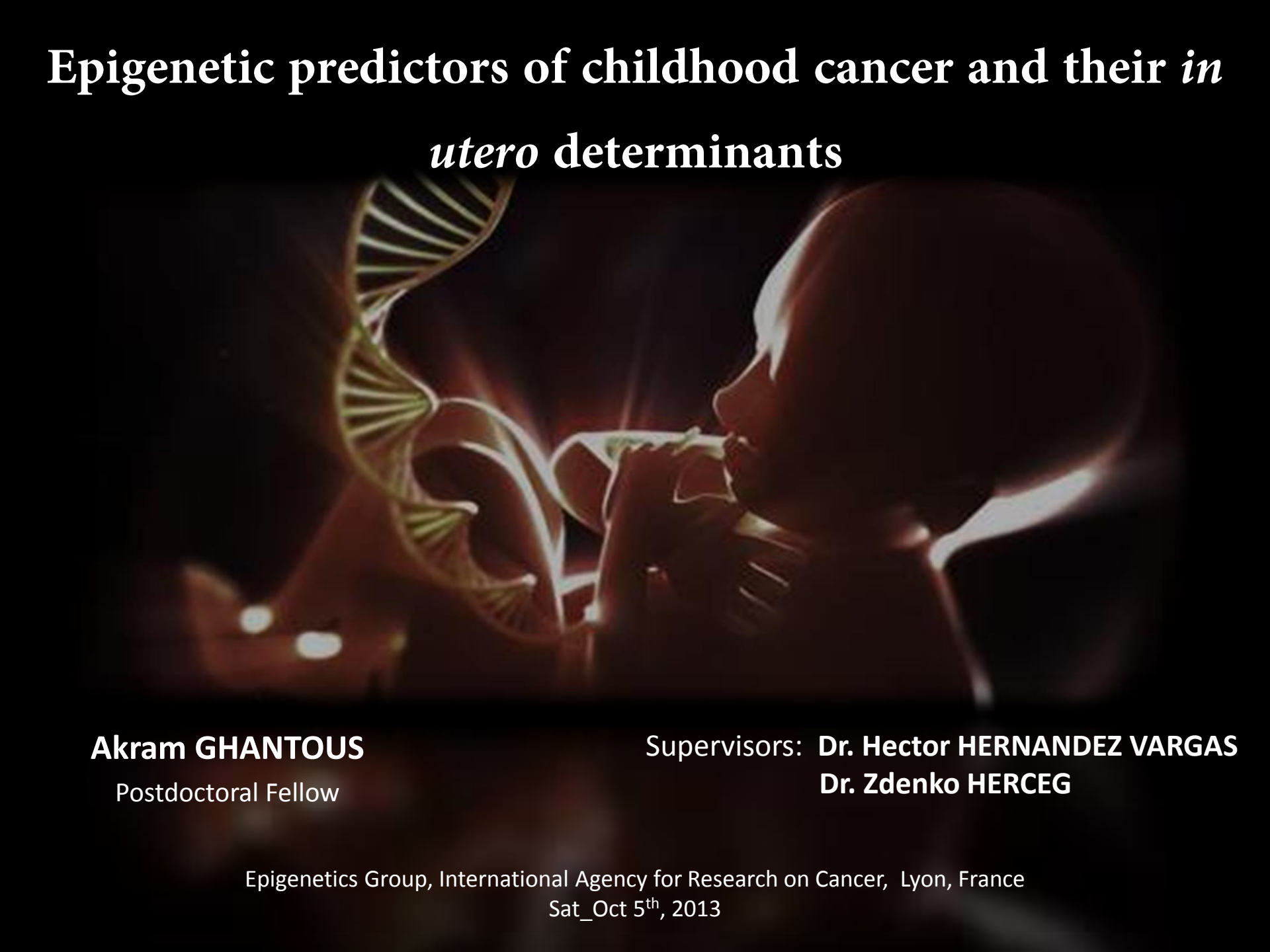
Epigenetic predictors of childhood cancer and their *in utero* determinants



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Dr. Zdenko HERCEG

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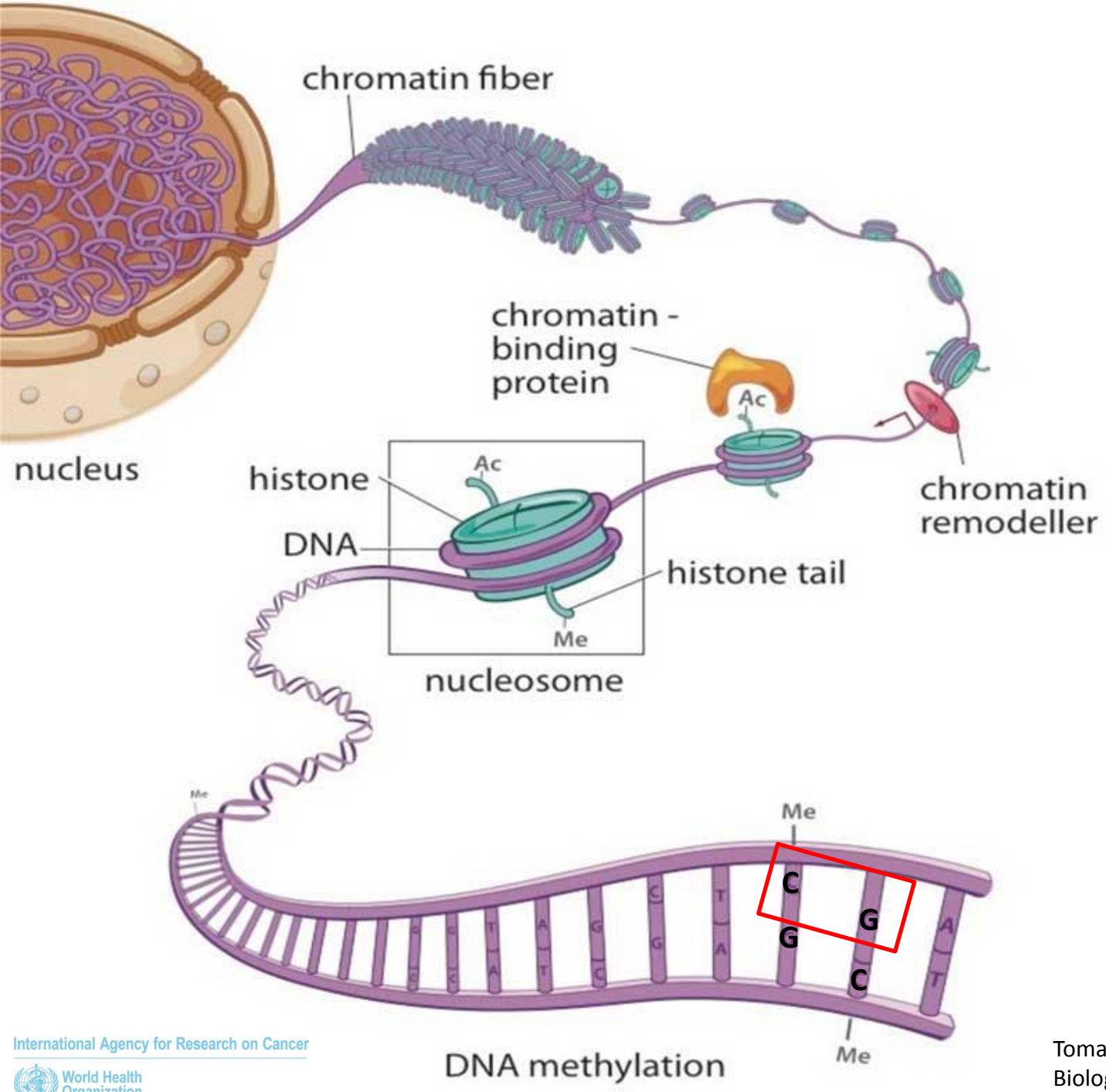
Sat_Oct 5th, 2013



Tomazou *et al.,* The Cell Biology of Stem Cells, 2010

Why Epigenetics?

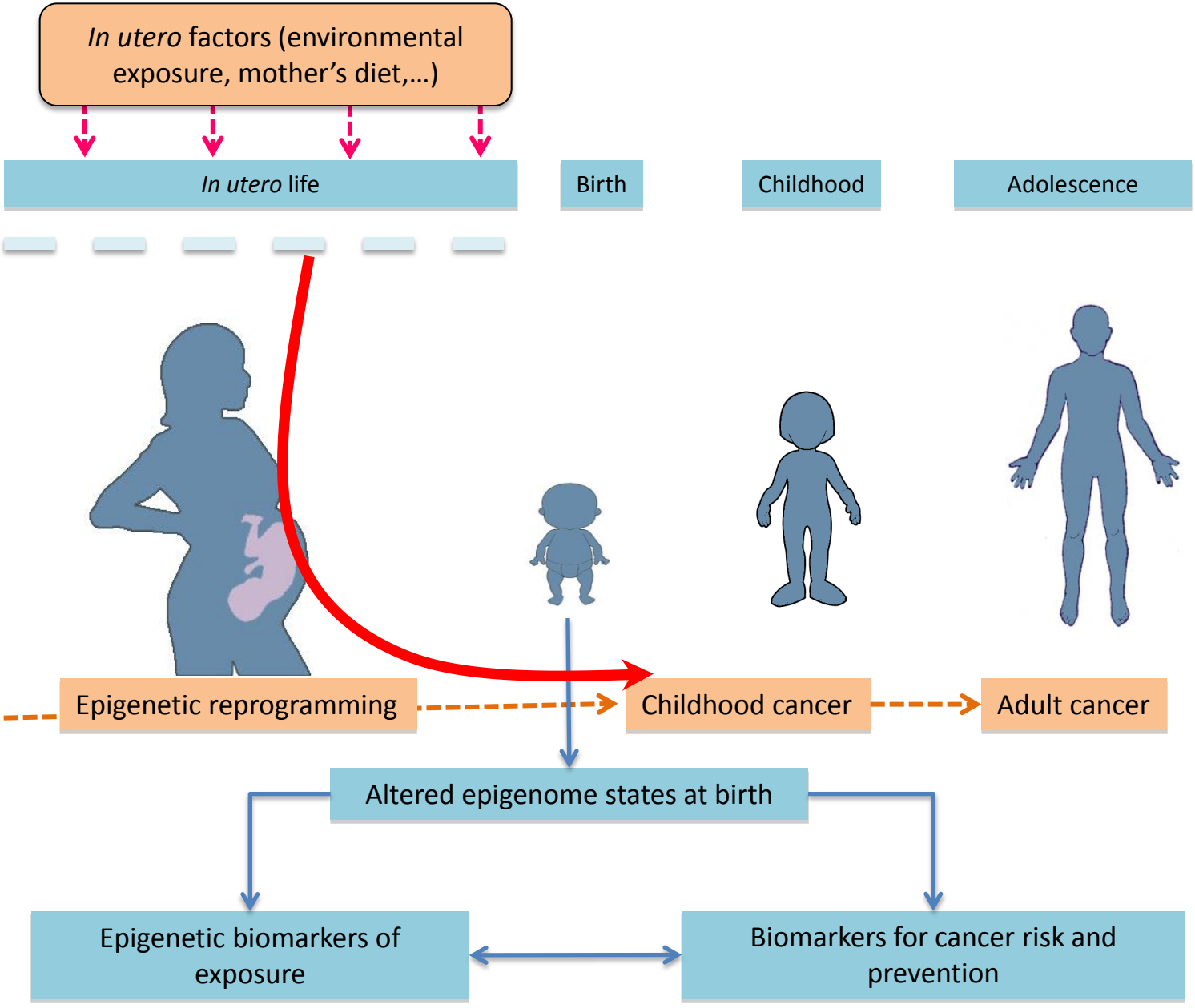
- DNA methylation marks are commonly deregulated in childhood cancers

DNA methylation is deregulated in ALL

MDR1, THSBS2, THSBS1, MYF3, ER, P15, CD10, c-ABL, p16, and *p73*

overrepresentation of Wnt-related genes

Evidence for prenatal origin of childhood cancer



The in utero environment-epigenetic-cancer associations have not been studied previously, because **biospecimens collected at birth**, **follow-up** and **sufficient sample size** are necessary.

Modified from Z. Herceg

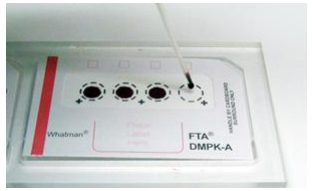
Cohort Profile: The International Childhood Cancer Cohort Consortium (I4C)

Rebecca C Brown,[1]* Terence Dwyer,[2] Carol Kasten,[3] Danuta Krotoski,[4] Zhu Li,[5] Martha S Linet,[3] Jørn Olsen,[6] Peter Scheidt[4] and Deborah M Winn[3] for the International Childhood Cancer Cohort Consortium (I4C)

International Journal of Epidemiology 2007;**36**:724–730

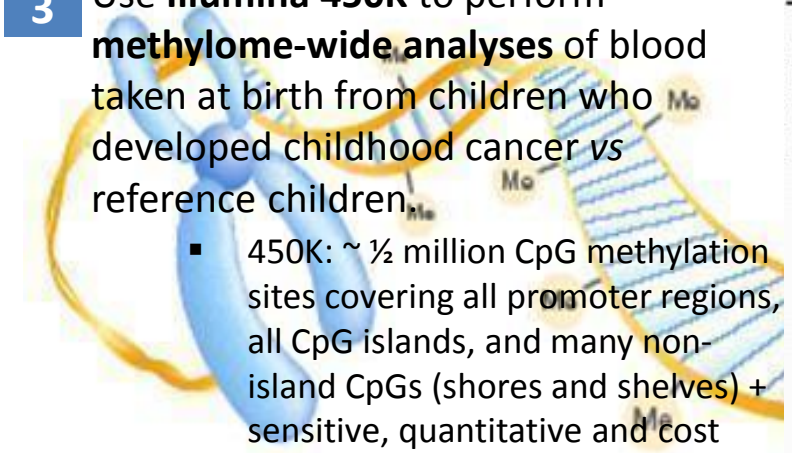
- National Children Study (**NCS**), USA
- Tasmanian Infant Health Study (**TIHS**), Aurstalia
- Norwegian Mother & Child Cohort Study (**MoBa**), Norway

AIMS



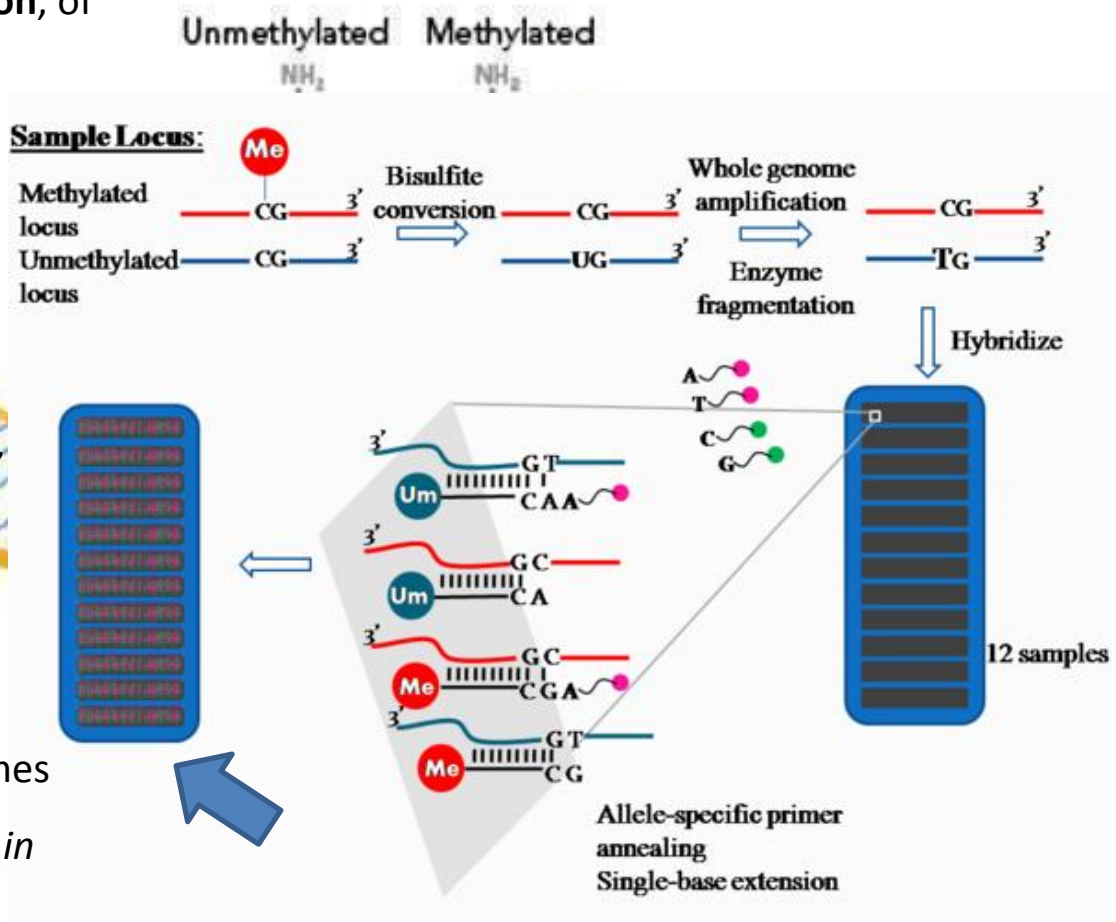
1 Most biospecimens from I4C are in the form of blood spots ⇨ Need to **optimize DNA extraction from blood spots**, which have <u>limited amounts of DNA</u>.

2 Optimize **bisulfite conversion** and, if needed, **whole bisulfitome amplification**, of DNA obtained from blood spots.



3 Use **Illumina 450K** to perform **methylome-wide analyses** of blood taken at birth from children who developed childhood cancer *vs* reference children.

- 450K: ~ ½ million CpG methylation sites covering all promoter regions, all CpG islands, and many non-island CpGs (shores and shelves) + sensitive, quantitative and cost effective



Frederick National Laboratory, NCI, USA

4 Develop and apply **bioinformatics** software pipelines to analyze methylomes

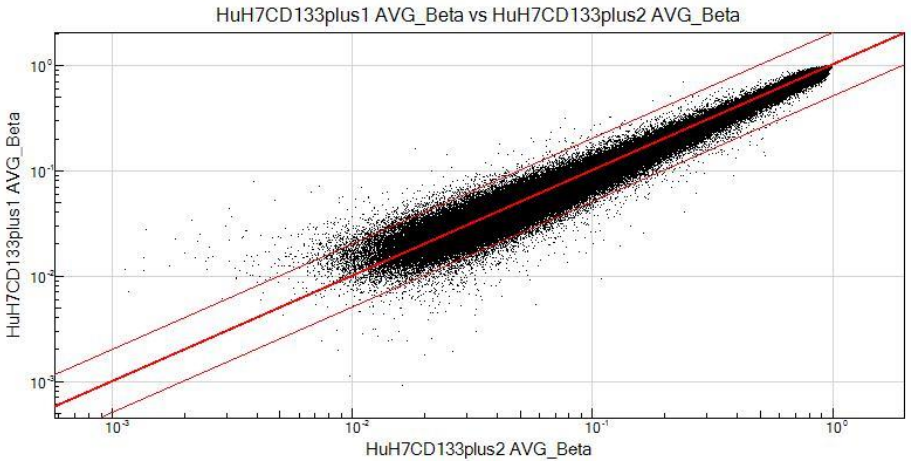
5 Decipher the mechanistic link between *in utero* exposure, methylome and childhood cancer

Amplifying DNA using Whole Bisulfitome Amplification (WBA) creates methylome bias

We used DNA from reference DNA from a cell line (enough DNA quantities)

Two batches of the same cell line:

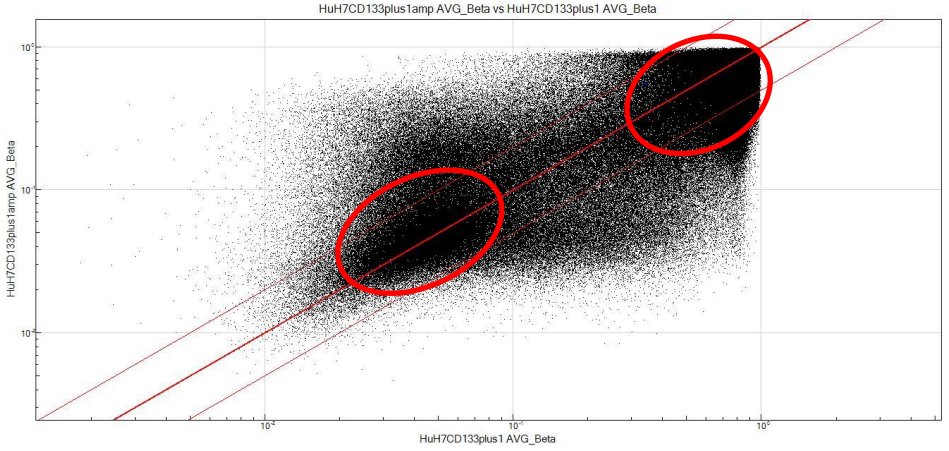
Batch 1 vs Batch 2



High correlation overall (r = 0 .985)

Amplified vs non-amplified batches of the same cell line:

Batch 1 **before** **vs** **after** WBA



Higher correlation in strongly hypomethylated and strongly hypermethylated regions than in between

Acquired by Genome Studio

Optimizing protocols to increase DNA yield Dried Blood Spots (DBS)

Less agitation time

Better protease

Milking by spin baskets

DNA

215 ng

85 ng

Undetectable by PCR

Type of column

Elute with high volumes,

then partially evaporate at 65°C

250 - 2500 ng

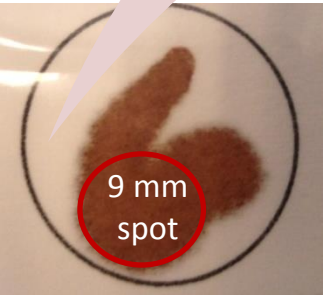
450K: require 4ul of 165ng

85 ng — Protease overnight

50 ng — Protease 2.5 h

<50 ng — Blood lysis solution vol is low (50 ul)

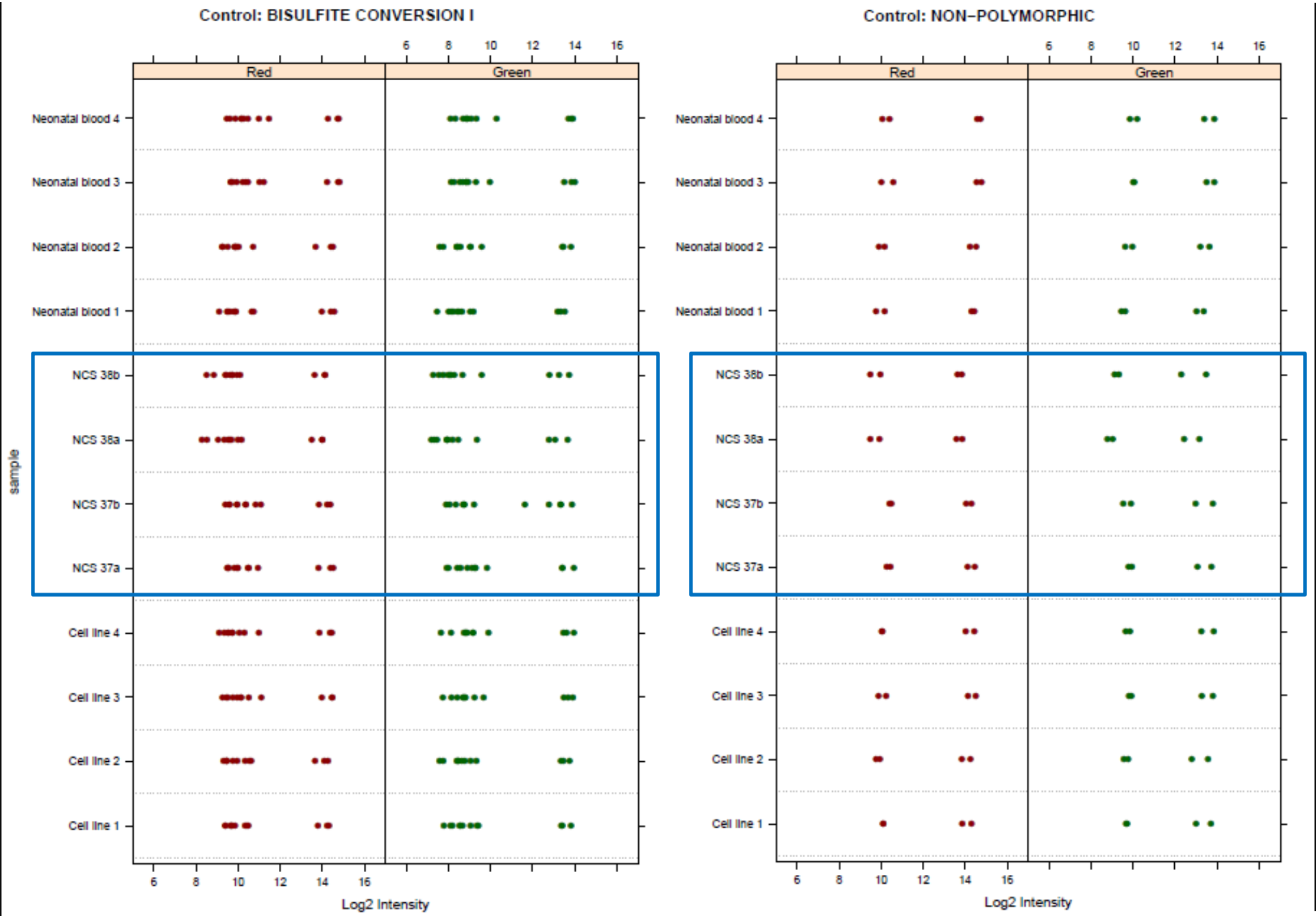
Both using 500 ul lysis soln (high vol)



A NCS spot is shown; objects drawn to scale

Not one single optimization step is needed, but multiple steps and kits to increase the DNA yield from undetectable levels to greater than 250 ng, enough for 450K methylome analysis.

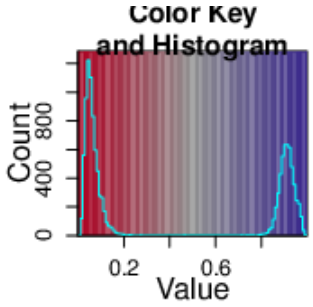
Blood Spots - 450K Methylome Analysis: Quality Controls

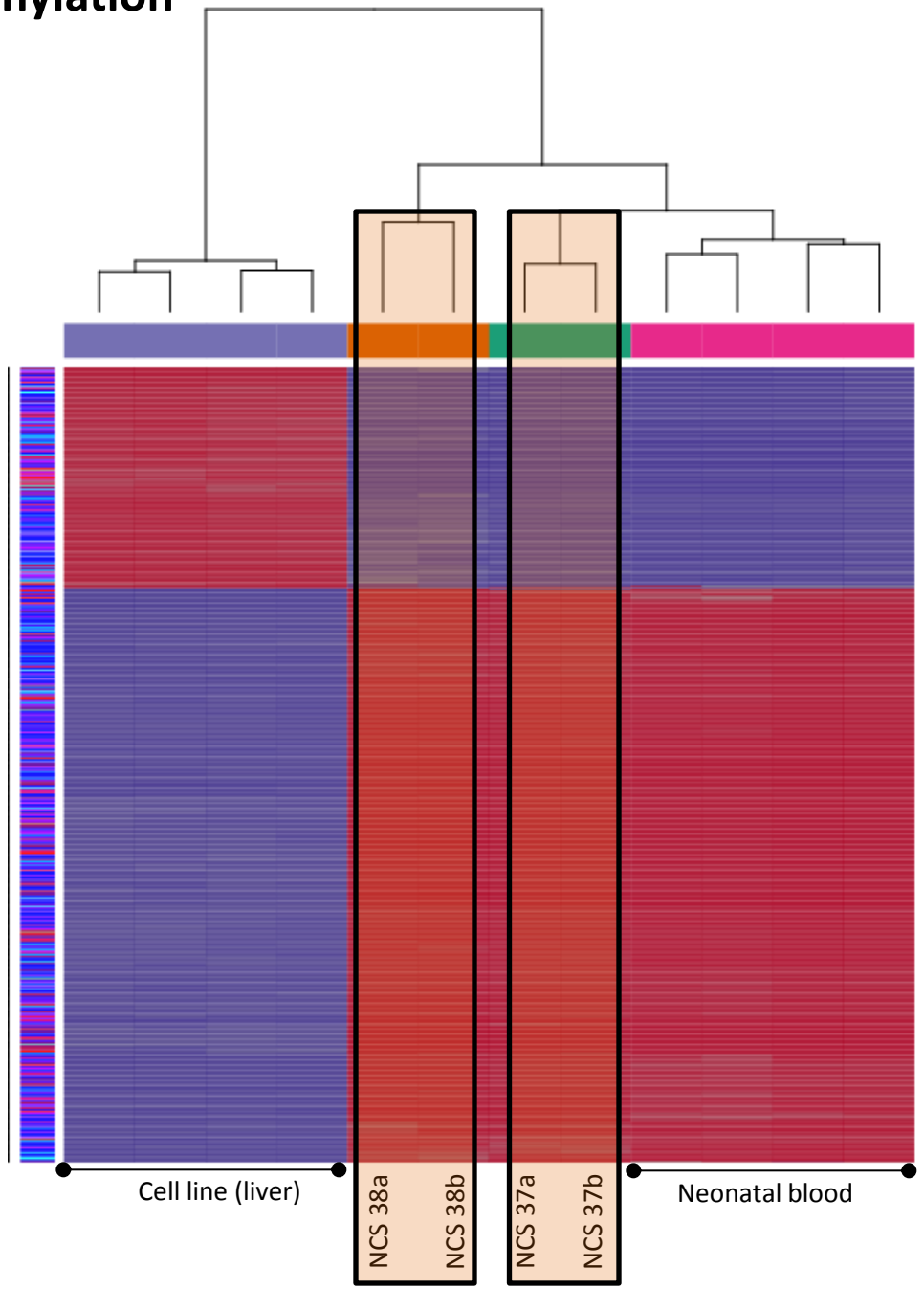


Blood Spots - Quality Control of Methylation Probes

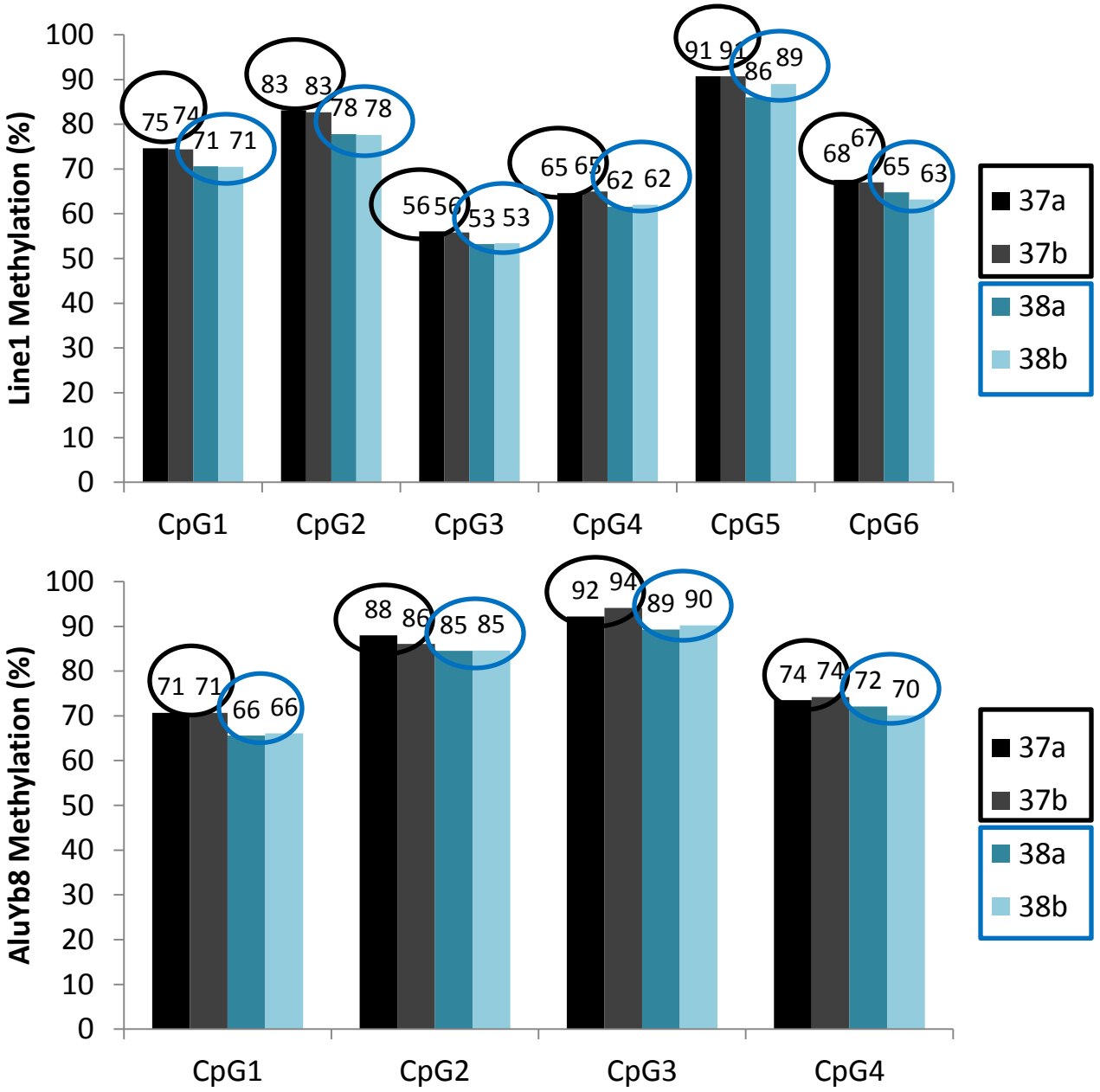
		Probe		Beta-value		
	Samples	**Detected CpGs (p<0.01)**	**CpG Percentage (p<0.01)**	**Average**	**Minimum**	**Maximum**
Neonatal Blood	NB 1672	485405	99.96	0.4886	0.0012	0.9929
	NB 1597	485392	99.96	0.4729	0.0006	0.9947
	NB 1842	485358	99.95	0.4911	0.0009	0.9940
	NB 1645	485119	99.91	0.4704	0.0011	0.9914
Blood Spots	NCS 37a	484990	99.88	0.4712	0.0038	0.9953
	NCS 37b	484946	99.87	0.4719	0.0045	0.9916
	NCS 38a	483897	99.65	0.4226	0.0005	0.9942
	NCS 38b	482519	99.37	0.4240	0.0001	0.9935
Cell Line	Cell Line 1	485124	99.91	0.4748	0.0022	0.9926
	Cell Line 2	485175	99.92	0.4813	0.0032	0.9920
	Cell Line 3	485342	99.95	0.4738	0.0006	0.9926
	Cell Line 4	485272	99.94	0.4743	0.0021	0.9934

Blood Spots: Differential Methylation by 450K

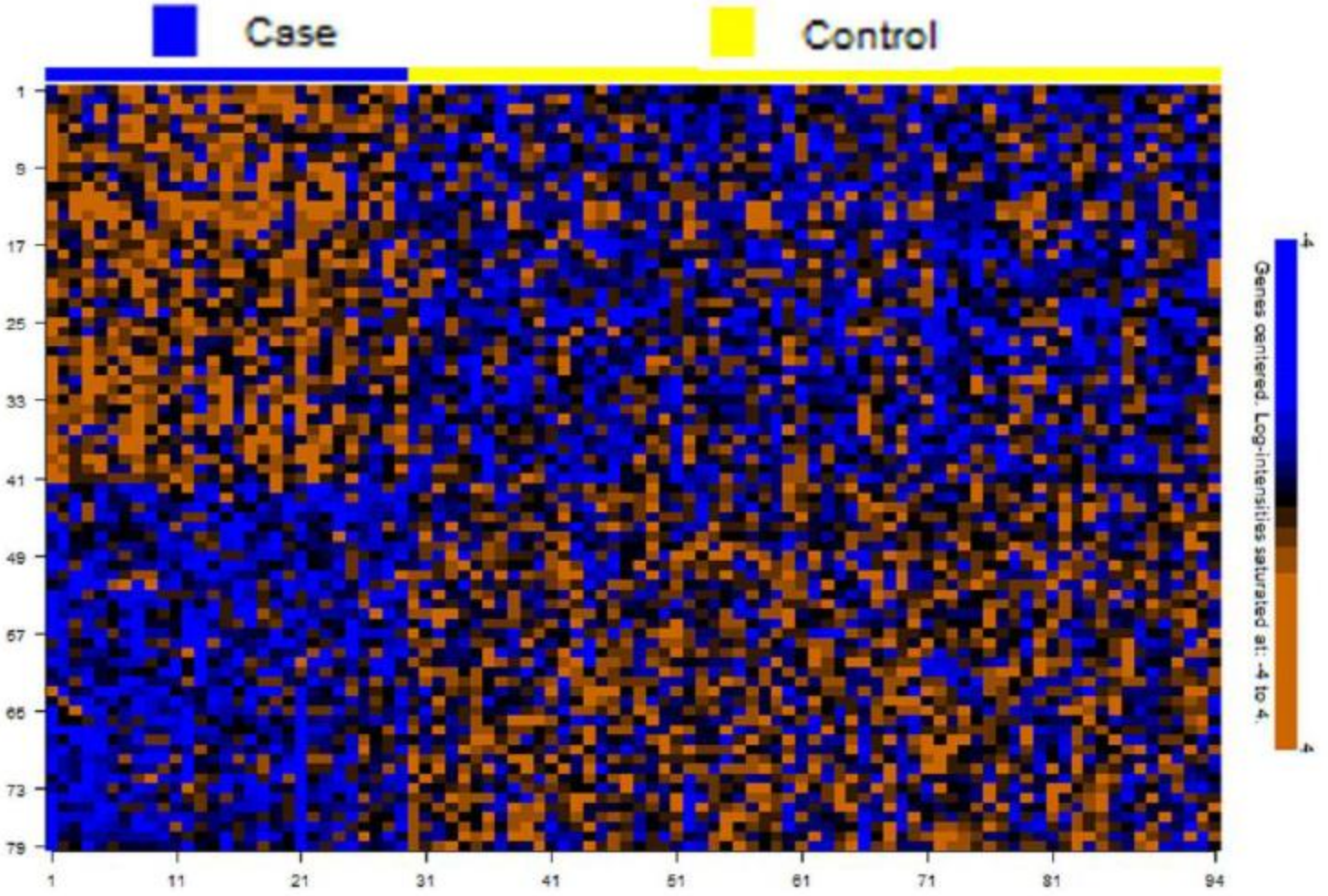




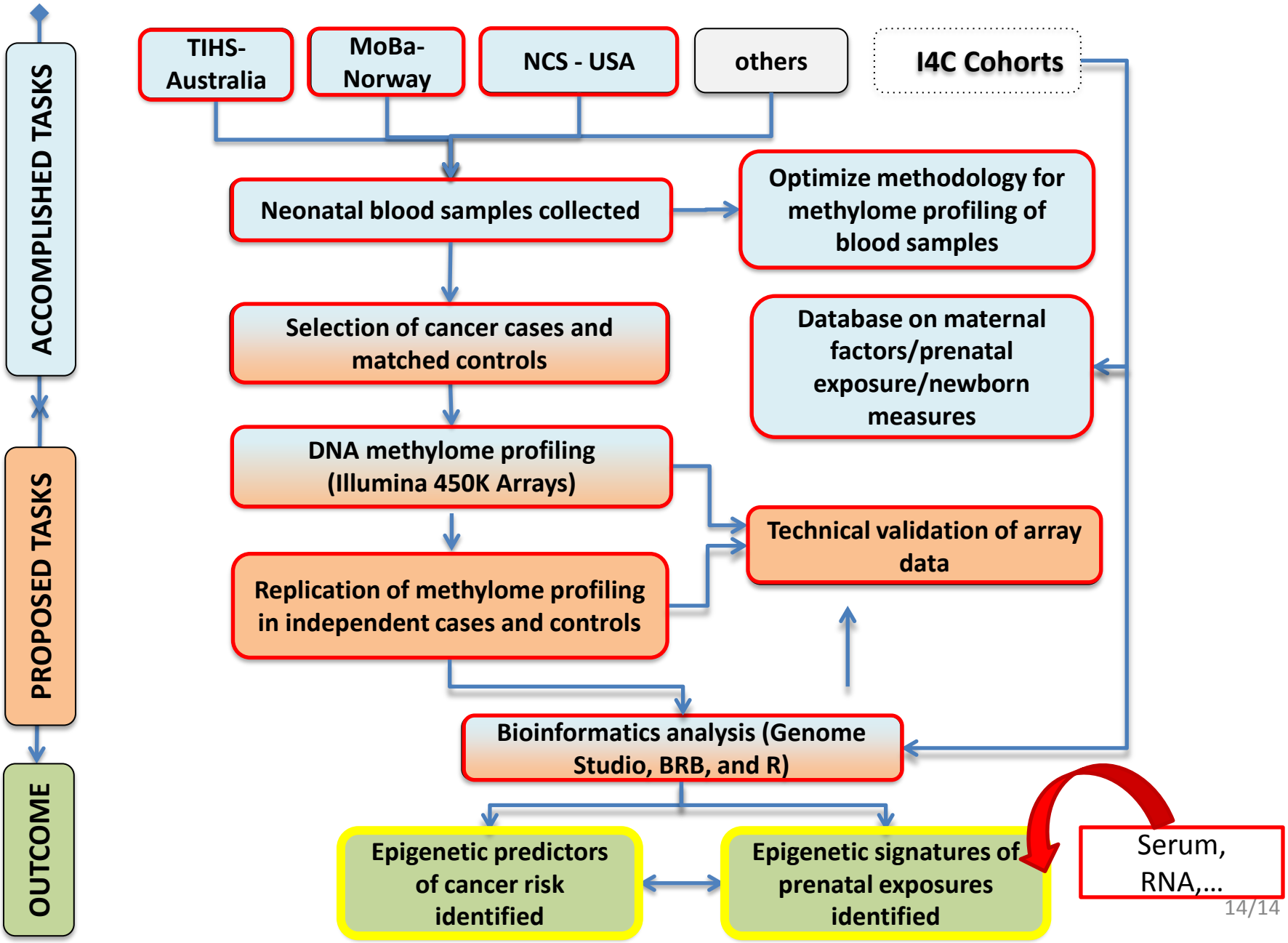
Blood Spots: Differential Methylation by Pyrosequencing



Cord Blood DNA (prospective): Differential Methylation by 450K



Summary and future perspectives



Conclusions

- **DNA Amplification using Whole Bisulfitome Amplification (WBA) introduces methylome bias.**
- **Up to 2500 ng of DNA with good quality can be extracted from a 9 mm blood spot, sufficient for methylome-wide and region-specific methylation studies.**
- **Whether provided as DNA freshly extracted from cord blood (freeze-stored) or given as dried blood spots (stored at room temperature), DNA from either of these 2 sources can be analyzed for methylome-wide and region-specific methylation using our platforms and tailored bioinformatic pipelines, while passing all 450K quality controls.**
- **The methylation signatures are reproducible among duplicates and consistent using several techniques of methylation analysis.**
- ***In utero* factors can cause changes in the methylome since birth.**